

§ 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments

In the Specification:

Please substitute the pending paragraph beginning on page 5, line 24, with the following paragraph:

c1
Figures 1A-1C show the nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of DR4. It is predicted that amino acids 1-23 constitute the signal peptide, amino acids 24-238 constitute the extracellular domain, amino acids 239-264 constitute the transmembrane domain, and amino acids 265-468 constitute the intracellular domain of which amino acids 379-422 constitute the death domain.

[Please substitute the pending paragraph beginning on page 5, line 29, with the following paragraph:

c2
Figures 2A-2C show the regions of similarity between the amino acid sequences of DR4, human tumor necrosis factor receptor 1 (SEQ ID NO:3), human Fas protein (SEQ ID NO:4), and the death domain containing receptor 3 (DR3) (SEQ ID NO:5).

Please substitute the pending paragraph beginning on page 7, line 10, with the following paragraph:

C3
Using the information provided herein, such as the nucleic acid sequence set out in Figures 1A-1C, a nucleic acid molecule of the present invention encoding a DR4 polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the gene of the present invention has also been identified in cDNA libraries of the following tissues: amniotic cells, heart, liver cancer, kidney, leukocyte, activated T-cell, K562 plus PMA, W138 cells, Th2 cells, human tonsils, and CD34 depleted buffy coat (cord blood).

Please substitute the pending paragraph beginning on page 7, line 18, with the following paragraph:

C4
The DR4 gene contains an open reading frame encoding a mature protein of about 445 amino acid residues whose initiation codon is at position 19-21 of the nucleotide sequence shown in Figures 1A-1C (SEQ ID NO.1), with a leader sequence of about 23 amino acid residues (i.e., a total protein length of 468 amino acids), and a deduced molecular weight of about 50 kDa. Of known members of the TNF receptor family, the DR4 polypeptide of the invention shares the greatest degree of homology with human TNFR1 and DR3 polypeptides shown in Fig. 2, including significant sequence homology over the multiple Cysteine Rich domains.

Please substitute the pending paragraph beginning on page 8, line 14, with the following paragraph:

C5
As indicated, the present invention also provides the mature form(s) of the DR4 protein of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the

cb
conf

mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species on the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequence encoding the mature DR4 polypeptide having the amino acid sequence encoded by the cDNA clones contained in the host identified as ATCC Deposit No. 97853, and as shown in Figures 1A-1C (SEQ ID NO:2). By the mature DR4 protein having the amino acid sequence encoded by the cDNA clones contained in the host identified as ATCC Deposit No. 97853, is meant the mature form(s) of the DR4 protein produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the clone contained in the vector in the deposited host. As indicated below, the mature DR4 having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853, may or may not differ from the predicted "mature" DR4 protein shown in Figure 1 (amino acids from about 24 to about 468) depending on the accuracy of the predicted cleavage site based on computer analysis.

[Please substitute the pending paragraph beginning on page 9, line 32, with the following paragraph:

cb

Isolated nucleic acid molecules of the present invention include DR4 DNA molecules comprising an open reading frame (ORF) shown in Figures 1A-1C (SEQ ID NO:1) and further include DNA molecules which comprise a sequence substantially different than all or part of the ORF whose initiation codon is at position 19-21 of the nucleotide sequence shown in Figures 1A-1C (SEQ ID NO:1) but which, due to the degeneracy of the genetic code, still encode the DR4 polypeptide or a fragment thereof. Of

cb
cont'd

course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

Please substitute the pending paragraph beginning on page 10, line 1, with the following paragraph:

C7

In another aspect, the invention provides isolated nucleic acid molecules encoding the DR4 polypeptide having an amino acid sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. 97853 on January 21, 1997. Preferably, these nucleic acid molecules will encode the mature polypeptide encoded by the above-described deposited cDNA clone. The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in Figures 1A-1C (SEQ ID NO:1) or the nucleotide sequence of the DR4 cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated DNA molecules and fragments thereof are useful as DNA probes for gene mapping by *in situ* hybridization of the DR4 gene in human tissue by Northern blot analysis.

Please substitute the pending paragraph beginning on page 10, line 12, with the following paragraph:

C8

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By fragments of an isolated DNA molecule having the nucleotide sequence shown in Figures 1A-1C (SEQ ID NO:1) are intended DNA fragments at least 20 bp, and more preferably at least 30 bp in length which are useful as DNA probes as discussed above. of course larger DNA fragments 50-1500 bp in length are also useful as DNA probes according to the present invention as are DNA fragments corresponding to most, if not all, of the nucleotide sequence shown in Figures 1A-1C (SEQ ID NO:1). By a fragment at least 20 bp in length, for example, is intended fragments which include 20 or more bases from the nucleotide sequence in Figures 1A-1C (SEQ ID NO:1).

Please substitute the pending paragraph beginning on page 10, line 22, with the following paragraph:

C9
Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising the DR4 extracellular domain (amino acid residues from about 24 to about 238 in Figure 1A (SEQ ID NO:2)); a polypeptide comprising the DR4 transmembrane domain (amino acid residues from about 239 to about 264 in Figure 1B (SEQ ID NO:2)); a polypeptide comprising the DR4 intracellular domain (amino acid residues from about 265 to about 468 in Figures 1B and 1C (SEQ ID NO:2)); and a polypeptide comprising the DR4 death domain (amino acid residues from about 379 to about 422 in Figure 1B (SEQ ID NO:2)). Since the location of these domains have been predicted by computer graphics, one of ordinary skill would appreciate that the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to 15 residues) depending on the criteria used to define the domain.

Please substitute the pending paragraph beginning on page 11, line 1, with the following paragraph:

C10
Preferred nucleic acid fragments of the present invention further include nucleic acid molecules encoding epitope-bearing portions of the DR4 protein. In particular, such nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about 35 to about 92 in Figure 1A (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 114 to about 160 in Figure 1A (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 169 to about 240 in Figure 1A (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 267 to about 298 in Figure 1B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 330 to about 364 in Figure 1B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 391 to about 404 in Figure 1B (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 418 to about 465 in Figures

c10
Cont'd

1B and 1C (SEQ ID NO:2). The inventors have determined that the above polypeptide fragments are antigenic regions of the DR4 protein. Methods for determining other such epitope-bearing portions of the DR4 protein are described in detail below.

[Please substitute the pending paragraph beginning on page 11, line 16, with the following paragraph:

c11

In addition, the invention provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO:1 as follows: HTOIY07R (SEQ ID NO:6) and HTXEY80R (SEQ ID NO:7) both shown in Figure 4.

[Please substitute the pending paragraph beginning on page 11, line 37, with the following paragraph:

c12

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in Figures 1A-1C (SEQ ID NO:1) or Figures 2A-2C (SEQ ID NO:3).

[Please substitute the pending paragraph beginning on page 12, line 3, with the following paragraph:

c13

Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3 terminal poly(A) tract of the DR4 cDNA shown in Figure 1C (SEQ ID NO:1)), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

75

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Please substitute the pending paragraph beginning on page 13, line 4, with the following paragraph:

C14

Further embodiments of the invention include isolated nucleic acid molecules that are at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical, to (a) a nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence in Figures 1A-1C (SEQ ID NO:2), including the predicted leader sequence; (b) nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence in Figures 1A-1C (SEQ ID NO:2), including the predicted leader sequence but lacking the amino terminal methionine; (c) a nucleotide sequence encoding the mature DR4 polypeptide (full-length polypeptide with the leader removed) having the amino acid sequence at positions about 24 to about 468 in Figures 1A-1C (SEQ ID NO:2); (d) a nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No. 97853; (e) a nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence including the leader but lacking the amino terminal methionine encoded by the cDNA clone contained in ATCC Deposit No. 97853; (f) a nucleotide sequence encoding the mature DR4 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853; (g) a nucleotide sequence that encodes the DR4 extracellular domain, (h) a nucleotide sequence that encodes the DR4 transmembrane domain, (i) a nucleotide sequence that encodes the DR4 intracellular domain, (j) a nucleotide sequence that encodes the DR4 death domain; or (k) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), (i), or (j) above.

Please substitute the pending paragraph beginning on page 14, line ³~~4~~ with the following paragraph:

C15

As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in

C15
Cont'd

Figures 1A-1C or to the nucleotide sequences of the deposited cDNA clone can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

Please substitute the pending paragraph beginning on page 14, line 18, with the following paragraph:

C16

The present application is directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figures 1A-1C (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNAs, irrespective of whether they encode a polypeptide having DR4 activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having DR4 activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having DR4 activity include, *inter alia*, (1) isolating the DR4 gene or allelic variants thereof in a cDNA library; (2) *in situ* hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the DR4 gene, as described in Verma *et al.*, *Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting DR4 mRNA expression in specific tissues.

[Please substitute the pending paragraph beginning on page 14, line 32, with the following paragraph:

c17
Preferred, however, are nucleic acid molecules having sequences at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figures 1A-1C (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNAs which do, in fact, encode a polypeptide having DR4 protein activity. By "a polypeptide having DR4 activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the DR4 protein of the invention (either the full-length protein or, preferably, the mature protein), as measured in a particular biological assay. For example, DR4 protein activity can be measured using the cell death assays performed essentially as previously described (A.M. Chinnaiyan, *et al.*, *Cell* 81, 505-12 (1995); M.P. Boldin, *et al.*, *J Biol Chem* 270, 7795-8 (1995); F.C. Kischkel, *et al.*, *EMBO* 14, 5579-5588 (1995); A.M. Chinnaiyan, *et al.*, *J Biol Chem* 271, 4961-4965 (1996)) or as set forth in Example 5, below. In MCF7 cells, plasmids encoding full-length DR4 or a candidate death domain containing receptors are co-transfected with the pLantern reporter construct encoding green fluorescent protein. Nuclei of cells transfected with DR4 will exhibit apoptotic morphology as assessed by DAPI staining. Similar to TNFR-1 and Fas/APO-1 (M. Muzio, *et al.*, *Cell* 85, 817-827 (1996); M. P. Boldin, *et al.*, *Cell* 85, 803-815 (1996); M. Tewari, *et al.*, *J Biol Chem* 270, 3255-60 (1995)), DR4-induced apoptosis is blocked by the inhibitors of ICE-like proteases, CrmA and z-VAD-fmk.

[Please substitute the pending paragraph beginning on page 15, line 13, with the following paragraph:

c18
Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of the deposited cDNA or the nucleic acid sequence shown in Figures 1A-1C (SEQ ID NO:1) will encode a polypeptide "having DR4 protein activity." In fact, since degenerate

c18
cont'd

variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having DR4 protein activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid).

Please substitute the pending paragraph beginning on page 21, line 23, with the following paragraph:

c19

The invention further provides an isolated DR4 polypeptide having the amino acid sequence shown in Figures 1A-1C [SEQ ID NO:2] or a peptide or polypeptide comprising a portion of the above polypeptides.

Please substitute the pending paragraph beginning on page 26, line 3, with the following paragraph:

c20

The polypeptides of the present invention also include the polypeptide encoded by the deposited cDNA including the leader, the mature polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein), the polypeptide of Figures 1A-1C (SEQ ID NO:2) including the leader, the polypeptide of Figures 1A-1C (SEQ ID NO:2) minus the amino terminal methionine, the polypeptide of Figures 1A-1C (SEQ ID NO:2) minus the leader, the extracellular domain, the transmembrane domain, the intracellular domain, the death domain, soluble polypeptides comprising all or part of the extracellular and intracellular domains but lacking the transmembrane domain as well as polypeptides which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptide encoded by the deposited cDNA clones, to the polypeptide of Figures 1A-1C (SEQ ID NO:2) and also

C20
Cont'd

include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

[Please substitute the pending paragraph beginning on page 26, line 31, with the following paragraph:

C21

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in Figures 1A-1C (SEQ ID NO:2) or to the amino acid sequence encoded by deposited cDNA clones can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

[Please substitute the pending paragraph beginning on page 27, line 4, with the following paragraph:

C22

The present inventors have discovered that the DR4 polypeptide is a 468 residue protein exhibiting three main structural domains. First, the ligand binding domain was identified within residues from about 24 to about 238 in Figures 1A and 1B [SEQ ID NO:2]. Second, the transmembrane domain was identified within residues from about 239 to about 264 in Figure 1B [SEQ ID NO:2]. Third, the intracellular domain was identified within residues from about 265 to about 468 in Figures 1B and 1C [SEQ ID NO:2]. Importantly, the intracellular domain includes a death domain at residues from about 379 to about 422. Further preferred fragments of the polypeptide shown in Figures 1A-1C [SEQ ID NO:2]

C22
cont'd

include the mature protein from residues about 24 to about 468 and soluble polypeptides comprising all or part of the extracellular and intracellular domains but lacking the transmembrane domain.

Please substitute the pending paragraph beginning on page 28, line 9, with the following paragraph:

C23

Non-limiting examples of antigenic polypeptides or peptides that can be used to generate DR4-specific antibodies include: a polypeptide comprising amino acid residues from about 35 to about 92 in Figure 1A (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 114 to about 160 in Figure 1A (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 169 to about 240 in Figures 1A and 1B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 267 to about 298 in Figure 1B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 330 to about 364 in Figure 1B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 391 to about 404 in Figures 1B and 1C (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 418 to about 465 in Figure 1C (SEQ ID NO:2). As indicated above, the inventors have determined that the above polypeptide fragments are antigenic regions of the DR4 protein.

In the Claims:

✓ Please cancel claims 22-83 without prejudice or disclaimer.

✓ Please add the following claims 84-248: